

Applicant : Martin F. Berry et al.
Serial No. : 09/447,023
Filed : November 22, 1999
Page : 3

Attorney's Docket No.: 00414-046001


50 (as amended) through 52;
58 through 62 (all amended);
63 through 68; and
70 through 84.

Applicants also include herein a copy of the Appendix from the parent application (which is incorporated by reference at page 1 of this application).

Kindly apply any amount due to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 10/23/00



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APPENDIX 1

TITLE: CRANBERRY PROCESSES AND PRODUCTS

APPLICANT: MARTIN F. BERRY, KATHERINE G. HAIGHT,
DONALD C. WEBER, HAROLD L. MANTIUS AND
LUTHER H. LEAKE

"EXPRESS MAIL" Mailing Label Number EL245470945US

Date of Deposit November 9, 1998

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Samara M. Scarlett
Samara M. Scarlett

Appendix 1

TITLE: Brix Measurement

DATE:

SUPERSEDES:

PAGE 1 of 2

I. Sample Preparation

- A. Sample shall be at ambient temperature when solids are read on refractometer.
- B. If sample is taken hot, place it in a stainless steel test tube, stopper on tube, and cool to approximately 70°F.
- C. For an approximate check, sample may be read hot off the line.

II. Procedure

- A. Bench Model Refractometer and Constant Temperature Water Bath or Temperature Compensating Refractometer.
 1. With a rubber policeman, place a small amount of sample on the bottom prism. (Never touch the prisms with a hard substance like glass, metal, or plastic. The prisms scratch easily and scratches result in a fuzzy line and a less accurate reading.)
 2. Gently close the hinged cover, turn field lamp on and adjust.
 3. Adjust the knob so that the demarcation line between the dark and light field cuts exactly through the intersection of the cross hairs. If digital model, read display and go to step 7.
 4. Depress the switch which turns the field lamp off and the internal scale lamp on.
 5. Read the bottom scale (° Brix) and also observe the prism temperature by means of the thermometer mounted on the side of the instrument.

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ab

6. Correct observed reading for temperature as necessary by consulting appropriate charts.
7. Finally, wash the prisms with distilled water and carefully dry with lens tissue or another very soft material. Refractometers are costly precision instruments and must be treated with care.

B. Hand Refractometer

1. Place a small amount of the sample on the prism and close hinged cover.
2. Hold instrument to a light source.
3. Note the point at which the dividing line crosses the scale, estimating to the nearest 0.1%.
4. Apply temperature corrections to this reading from thermometer scale at side of refractometer.
5. Cleaning - Wash the prism end of the refractometer carefully using lukewarm water only. Then dry with lens tissue or other soft tissue so that the prism surface is not scratched. Refractometers are costly precision instruments and must be treated with care.

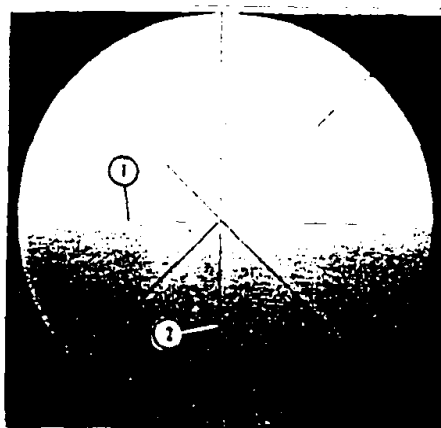
NOTE: Refractometers should be serviced periodically to ensure proper operation. See owner's manual for service schedule.

ATTACHMENT 1

International Temperature Correction Table for the Normal Model of Refractometer Above and Below 20°C.																
Temp. ° C.	Per cent Sucrose															
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	
	Subtract from the per cent Sucrose															
10	0.50	0.54	0.58	0.61	0.64	0.66	0.68	0.70	0.72	0.73	0.74	0.75	0.76	0.78	0.79	
11	0.46	0.49	0.53	0.55	0.58	0.60	0.62	0.64	0.65	0.66	0.67	0.68	0.69	0.70	0.71	
12	0.42	0.45	0.48	0.50	0.52	0.54	0.56	0.57	0.58	0.59	0.60	0.61	0.61	0.63	0.63	
13	0.37	0.40	0.42	0.44	0.46	0.48	0.49	0.50	0.51	0.52	0.53	0.54	0.54	0.55	0.55	
14	0.33	0.35	0.37	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.45	0.46	0.46	0.47	0.48	
15	0.27	0.29	0.31	0.33	0.34	0.34	0.35	0.36	0.37	0.37	0.38	0.39	0.39	0.40	0.40	
16	0.22	0.24	0.25	0.26	0.27	0.28	0.28	0.29	0.30	0.30	0.30	0.31	0.31	0.32	0.32	
17	0.17	0.18	0.19	0.20	0.21	0.21	0.21	0.22	0.22	0.23	0.23	0.23	0.23	0.24	0.24	
18	0.12	0.13	0.13	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.16	
19	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	
Add to the per cent Sucrose																
21	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	
22	0.13	0.13	0.14	0.14	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.16	0.16	
23	0.19	0.20	0.21	0.22	0.22	0.23	0.23	0.23	0.23	0.24	0.24	0.24	0.24	0.24	0.24	
24	0.26	0.27	0.28	0.29	0.30	0.30	0.31	0.31	0.31	0.31	0.31	0.32	0.32	0.32	0.32	
25	0.33	0.35	0.36	0.37	0.38	0.38	0.39	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	
26	0.40	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	
27	0.48	0.50	0.52	0.53	0.54	0.55	0.55	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	
28	0.56	0.57	0.60	0.61	0.62	0.63	0.63	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.64	
29	0.64	0.66	0.68	0.69	0.71	0.72	0.72	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	
30	0.72	0.74	0.77	0.78	0.79	0.80	0.80	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	

FIGURE 1 View of Reflection
Borderline

Total Reflection Borderline
Dual Reticle



Manual Bench Refractometer
Section 5.5.1

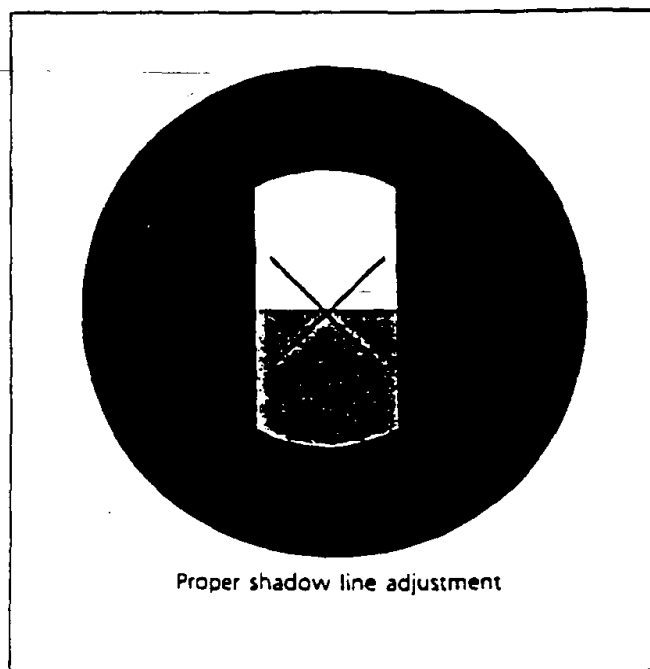


Figure 2

Digital Bench Refractometer
Section 5.5.4

APPENDIX 2

TITLE: CRANBERRY PROCESSES AND PRODUCTS

APPLICANT: MARTIN F. BERRY, KATHERINE G. HAIGHT,
DONALD C. WEBER, HAROLD L. MANTIUS AND
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Katharine M. Seabolt
Harold L. Mantius

Determination of the Total Anthocyanin Content (TAcv) and
Pigment Degradation Index (PDI) of Cranberry Products

I. Principle

This procedure is a pH differential method utilizing buffer solutions at pH 1.0 and pH 4.5. Absorbances are measured at 510 nm. TAcv and PDI are then calculated from both the O.D.'s and the extinction coefficients that have been established for the dissolved anthocyanins in the buffers. A procedure for determining TAcv content only in undegraded samples is also included.

II. Apparatus

- a) High speed centrifuge (Dupont Instruments-Sorvall Model SS-3 Automatic Centrifuge w/SS-34 rotor and 50 ml plastic centrifuge tubes)
- b) 5 and 10 ml autopipets
- c) 25 ml volumetric flasks
- d) Perkin-Elmer Model 552 spectrophotometer or Bausch & Lomb Spectronic 20 colorimeter
- e) 5 liter volumetric flask
- f) Two, 2 gallon polyethylene jugs to hold buffer solutions
- g) Whatman glass microfiber filters, type 934-AH, 11.0 cm diameter

III. Preparation of Buffers

pH 1.0 buffer:

Dilute the following with water to 5 liters (have 1 to 2 liters of water in the 5 liter volumetric flask before acid is added):

20.3 gms of KCl

60 mls of concentrated (12N) HCl

Make sure final pH of buffer is 0.80-0.90.

pH 4.5 buffer:

Dilute the following with water to 5 liters (have 1 to 2 liters of water in the 5 liter volumetric flask before acid is added):

425.5 gms of sodium acetate $3H_2O$

90-100 mls of concentrated (12N) HCl

Make sure final pH of buffer is 4.8-4.9.

Multiply absorbance of pH 4.5 buffered sample by **conversion factor** to yield "Total O.D. 4.5".

Determine Total Anthocyanin Content (TAcy) as follows:

$$\text{TAcy} = \frac{\text{Total O.D. 1.0} - \text{Total O.D. 4.5}}{77.5} = \frac{\text{mg TAcy}}{100 \text{ ml}}$$

Note: The TAcy content is for 100 ml of the sample that was added to the 25 ml flasks so if a preliminary dilution occurred (Table 1), then this dilution has to be corrected for when the TAcy content of the starting material is desired .

Determine Pigment Degradation Index (PDI) as follows:

$$\text{PDI} = \frac{\text{Total O.D. 1.0} / 87.3}{(\text{Total O.D. 1.0} - \text{Total O.D. 4.5}) / 77.5}$$

Determination of TAcy Content of Cranberry Products without PDI being Determined

Procedure

Follow the same procedure for determining TAcy and PDI (Section IV.) except do not dilute sample with pH 4.5 buffer. Use the same dilutions for the pH 1.0 buffer listed in Table 1.

$$\text{TAcy(mg/100 ml)} = \frac{\text{O.D. of pH 1.0 buffered sample} \times \frac{\text{size of ml of sample added}}{\text{vol. flask}} \times \frac{100}{87.3}}{\text{to vol. flask}}$$

Note: As previously noted, the TAcy content is for 100 mls of sample that was added to the 25 ml flasks so that if TAcy content of the starting material is desired any preliminary dilutions have to be corrected for.

Procedure:

Clarify samples by centrifuging for 10 minutes at 15,000 rpm in 50 ml plastic centrifuge tubes using SS-34 rotor. If sample does not contain cranberry juice at 2.0°Bx (i.e., is not Cocktail or CRANAPPLE) then see Table 1 for correct dilution, otherwise proceed. Pipet (using 10 ml autopipet) 10 mls of centrifuged sample into a 25 ml volumetric flask. Be careful not to disturb pellet of insolubles at bottom of centrifuge tube. Dilute to the mark with pH 4.5 buffer. Pipet (using 5 ml pipet) 2.5 ml of remaining centrifuged sample into a 25 ml volumetric flask. Dilute to the mark with pH 1.0 buffer. Mix buffered samples well by inverting flasks several times. Store the buffered solutions in the dark for approximately 2 hours. After insuring that solutions are thoroughly mixed, measure the absorbance at 510 nm using distilled water as a blank. Use 1 cm square cells with Perking-Elder or 1.13 cm I.D. tubes with Bausch & Lomb. Divide Bausch & Lomb absorbances by 1.13 to convert absorbances to that of a 1 cm cell.

Table 1

Sample preparation of various cranberry materials prior to TAcv and PDI determination.

Cranberry material	Preliminary dilution	ml to add to 25 ml flask pH 1.0	ml to add to 25 ml flask pH 4.5
single strength juice	none	0.5	2.5
leach water	none	2.5	10.0
50°bx Conc.	1.7 ml of conc. to 50 ml w/ dH ₂ O	2.5	10.0
Jellied sauce	Blend 100 g + 100 ml dH ₂ O, filter thru glass microfiber filter.	2.5	10.0
Cranberry puree	25 g centrifuge for 10 min at 15,000 RPM.	1.0	2.5

Calculations for TAcv and PDI

Conversion factor = (size of vol. flask) x (100/ml of sample added to vol. flask)

Multiply absorbance of pH 1.0 buffered sample by conversion factor to yield "Total O.D. 1.0".

APPENDIX 3

TITLE: CRANBERRY PROCESSES AND PRODUCTS

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Thomas M. Scarlett
THOMAS M. SCARLETT

33-29-27,

2-50A or

.09-873G,
NO. HT-4100

at
her/Wheaton

1991 FISHER
sewhere for

RY TWO WEEKS.
VG SEASON, AND
GHT on blender
ed through Faring
003540 - \$2.40 each
003515 - \$3.00

YPAC
G ORDER)

(vent.)

- 3.1 Using balance, weight out $100 \text{ gm} \pm .10$ of fresh fruit and transfer to clean blender jar. Transfer 120 ml of 0.2 NORMAL HYDROCHLORIC ACID AQUEOUS EXTRACTION SOLVENT into 250 ml GRADUATED CYLINDER by using 100 ml DISPENSETTE BOTTLE TOP DISPENSER, where delivery volume is set at 60 ml. Pump twice. Pour cylinder contents into blender jar. To avoid sample splattering onto jar lid and sides initially start blender at low or medium speed via variable voltage transformer for first 5-10 seconds followed by full speed blending for 3 minutes.
- 3.2 While the blender runs:
 - a) Put GLASS MICROFIBER FILTER DISC with swirling side up in Coor Porcelain Buchner funnel; put funnel on the 500 ml vacuum flask.
 - b) Using 50 ml DISPENSETTE BOTTLE TOP DISPENSER, measure out 50 ml of AQUEOUS EXTRACTION SOLVENT into 50 ml graduated cylinder. Pour 2-5 ml of this solvent onto the filter disc to moisten filter. No suction.
 - c) Weigh 100 gm of berries for the next extraction.
 - d) Measure out 120 ml of AQUEOUS EXTRACTION SOLVENT for next assay into the 250 ml GRADUATED CYLINDER.
- 3.3 When blending is finished AND BLENDER HAS STOPPED FULLY, remove jar from blender base and remove cover from jar.
- 3.4 Swirl contents then immerse 10 ml wide mouth pipet tip into the slurry. Rinse pipet with slurry 2-3 times using safety bulb. Fill pipet above 0-mark and then adjust to 10 ml.
- 3.5 Hold pipet over funnel and empty pipet using the safety bulb to push out the slurry twice onto the filter, quickly.
- 3.6 Attach suction flask to the pump (which is turned on) and pour remainder of the 50 ml AQUEOUS EXTRACTION SOLVENT at the same time (perhaps a fraction of a second later, not sooner) into funnel. SWIRL CONTENTS IN FUNNEL DURING FILTERING TO GET SLURRY AND SOLVENT INTO SUSPENSION.
- 3.7 Dilution and measurement of absorption: Into a dry cuvette measure 0.8 ml of filtrate with EPENDORF MICROPIPET, and add 4.0 ml of AQUEOUS EXTRACTION SOLVENT with the 5 ML REPIPET DISPENSER set at 4.0 ml, invert to mix. Wipe cuvet with kim-wipe and insert into sample compartment to determine the absorbance @515nm. Refer to 1991 Aqueous TAcY extraction chart to determine TAcY and record. TAcY can also be calculated with this formula $TAcY = (\text{absorbance} + 0.0086) - 0.008$.
- 3.8 Standardize Hach and cuvettes according to those attached procedures. The dispensers and Eppendorf pipet must be standardized daily by delivering into appropriate calibrated volumetric vessels to make sure they are dispensing exactly 120, 50, 4.0 and 0.8 mls.
- 3.9 Pour contents of suction flask and sample cuvette into blender jar containing remaining slurry. Add 68 mls of 1 normal potassium hydroxide to blender jar contents by using 100 ml dispensette bottle top dispenser where delivery volume is set at 68 ml. Blend jar contents until well mixed (approximately 10-15 seconds). Once per day check pH of one blender jar using litmus paper. If necessary, adjust potassium hydroxide volume to maintain pH between 5.5 and 8.0. Dispose of slurry as recommended for your receiving station.
- 3.10 Rinse blender jar, suction flask, and sample cuvette before next assay.
- 3.11 Filter paper may be disposed of as normal paper waste.

- 1) Turn power to the unit on by pressing I/O key. The instrument performs a self test. Set the 515 NM wavelength and allow for a 15 (+) minute warm-up, prior to use.
- 2) Place the 1.13 cm adapter (part #44798-00 or #44799-00 with insert) in the sample compartment, with the window open left to right..
- 3) The instrument will display **Enter Program #**. To place the instrument in absorbance reading mode Press "0" and "ENTER". To put the instrument in constant on mode press "SHIFT:", "SETUP" or "2" and the "down arrow" twice. The screen displays **Lamp** flashing: **Momentary** not flashing. Press "ENTER" the **Momentary** flashes, press the "down arrow" once to make **constant on** appear and flash, press "ENTER" and then "EXIT".
- 4) Match two sample cuvettes. Fill two clean cuvettes with aqueous extraction solvent. Place one cuvette in the sample compartment and cover, call this the reference cuvette. Press the zero key. Place the other cuvette in the sample compartment and cover. Read the displayed absorbance. If less than 0.003, the cuvettes are matched. Otherwise, repeat the second step, trying different cuvettes.
- 5) The unit is zeroed or re-zeroed, in the manner described above for matching sample cuvettes, utilizing the aqueous extraction solvent filled reference cuvette. There is generally no need to re-zero the unit between sample readings. However, if changes in the reference solution are suspected, or the unit has been turned off at all, or drift is suspected, it should always be re-zeroed.
- 6) When reading sample absorbances, observe the reading for a few moments (5-10 seconds) to insure that it has stabilized.
- 7) For optimum instrument performance and reproducibility, the following should be considered:
 - a) Set the wavelength by approaching the desired value from the counter clockwise direction, higher to lower wavelength.
 - b) Perform lamp calibration adjustment and lamp accuracy test weekly.

Equivalent TAcY Pigment Values vs Values in mg TAcY/100 gm

Absorbance Readings @ 515 nm

ABS	TAcY	ABS	TAcY	ABS	TAcY	ABS	TAcY	ABS	TAcY	ABS	TAcY
0.025 = 3		0.125 = 15		0.225 = 26		0.325 = 38		0.425 = 49		0.525 = 61	
0.030 = 3		0.130 = 15		0.230 = 27		0.330 = 38		0.430 = 50		0.530 = 62	
0.035 = 4		0.135 = 16		0.235 = 27		0.335 = 39		0.435 = 51		0.535 = 62	
0.040 = 5		0.140 = 16		0.240 = 28		0.340 = 40		0.440 = 51		0.540 = 63	
0.045 = 5		0.145 = 17		0.245 = 28		0.345 = 40		0.445 = 52		0.545 = 63	
0.050 = 6		0.150 = 17		0.250 = 29		0.350 = 41		0.450 = 52		0.550 = 64	
0.055 = 6		0.155 = 18		0.255 = 30		0.355 = 41		0.455 = 53		0.555 = 65	
0.060 = 7		0.160 = 19		0.260 = 30		0.360 = 42		0.460 = 53		0.560 = 65	
0.065 = 8		0.165 = 19		0.265 = 31		0.365 = 42		0.465 = 54		0.565 = 66	
0.070 = 8		0.170 = 20		0.270 = 31		0.370 = 43		0.470 = 55		0.570 = 66	
0.075 = 9		0.175 = 20		0.275 = 32		0.375 = 44		0.475 = 55		0.575 = 67	
0.080 = 9		0.180 = 21		0.280 = 33		0.380 = 44		0.480 = 56		0.580 = 67	
0.085 = 10		0.185 = 22		0.285 = 33		0.385 = 45		0.485 = 56		0.585 = 68	
0.090 = 10		0.190 = 22		0.290 = 34		0.390 = 45		0.490 = 57		0.590 = 69	
0.095 = 11		0.195 = 23		0.295 = 34		0.395 = 46		0.495 = 58		0.595 = 69	
0.100 = 12		0.200 = 23		0.300 = 35		0.400 = 47		0.500 = 58		0.600 = 70	
0.105 = 12		0.205 = 24		0.305 = 35		0.405 = 47		0.505 = 59		0.605 = 70	
0.110 = 13		0.210 = 24		0.310 = 36		0.410 = 48		0.510 = 59		0.610 = 71	
0.115 = 13		0.215 = 25		0.315 = 37		0.415 = 48		0.515 = 60		0.615 = 72	
0.120 = 14		0.220 = 26		0.320 = 37		0.420 = 49		0.520 = 60		0.620 = 72	

TAcY can be calculated by the following formula $TAcY = (Absorbance / 0.0086) - 0.008$

Values in mg TAcY/100 gm

Absorbance Readings @ 515 nm

ABS	Tacy	ABS	Tacy	ABS	Tacy	ABS	Tacy	ABS	Tacy	ABS	Tacy	ABS	Tacy	ABS	Tacy	ABS	Tacy	ABS	Tacy	ABS	Tacy
0.025 = 3		0.125 = 15		0.225 = 26		0.325 = 38		0.425 = 49		0.525 = 61		0.625 = 73		0.725 = 84		0.825 = 96		0.925 = 108			
0.030 = 3		0.130 = 15		0.230 = 27		0.330 = 38		0.430 = 50		0.530 = 62		0.630 = 73		0.730 = 85		0.830 = 97		0.930 = 108			
0.035 = 4		0.135 = 16		0.235 = 27		0.335 = 39		0.435 = 51		0.535 = 62		0.635 = 74		0.735 = 85		0.835 = 97		0.935 = 109			
0.040 = 5		0.140 = 16		0.240 = 28		0.340 = 40		0.440 = 51		0.540 = 63		0.640 = 74		0.740 = 86		0.840 = 98		0.940 = 109			
0.045 = 5		0.145 = 17		0.245 = 28		0.345 = 40		0.445 = 52		0.545 = 63		0.645 = 75		0.745 = 87		0.845 = 98		0.945 = 110			
0.050 = 6		0.150 = 17		0.250 = 29		0.350 = 41		0.450 = 52		0.550 = 64		0.650 = 76		0.750 = 87		0.850 = 99		0.950 = 110			
0.055 = 6		0.155 = 18		0.255 = 30		0.355 = 41		0.455 = 53		0.555 = 65		0.655 = 76		0.755 = 88		0.855 = 99		0.955 = 111			
0.060 = 7		0.160 = 19		0.260 = 30		0.360 = 42		0.460 = 53		0.560 = 65		0.660 = 77		0.760 = 88		0.860 = 100		0.960 = 112			
0.065 = 8		0.165 = 19		0.265 = 31		0.365 = 42		0.465 = 54		0.565 = 66		0.665 = 77		0.765 = 89		0.865 = 101		0.965 = 112			
0.070 = 8		0.170 = 20		0.270 = 31		0.370 = 43		0.470 = 55		0.570 = 66		0.670 = 78		0.770 = 90		0.870 = 101		0.970 = 113			
0.075 = 9		0.175 = 20		0.275 = 32		0.375 = 44		0.475 = 55		0.575 = 67		0.675 = 78		0.775 = 90		0.875 = 102		0.975 = 113			
0.080 = 9		0.180 = 21		0.280 = 33		0.380 = 44		0.480 = 56		0.580 = 67		0.680 = 79		0.780 = 91		0.880 = 102		0.980 = 114			
0.085 = 10		0.185 = 22		0.285 = 33		0.385 = 45		0.485 = 56		0.585 = 68		0.685 = 80		0.785 = 91		0.885 = 103		0.985 = 115			
0.090 = 10		0.190 = 22		0.290 = 34		0.390 = 45		0.490 = 57		0.590 = 69		0.690 = 80		0.790 = 92		0.890 = 103		0.990 = 115			
0.095 = 11		0.195 = 23		0.295 = 34		0.395 = 46		0.495 = 58		0.595 = 69		0.695 = 81		0.795 = 92		0.895 = 104		0.995 = 116			
0.100 = 12		0.200 = 23		0.300 = 35		0.400 = 47		0.500 = 58		0.600 = 70		0.700 = 81		0.800 = 93		0.900 = 105		1.000 = 116			
0.105 = 12		0.205 = 24		0.305 = 35		0.405 = 47		0.505 = 59		0.605 = 70		0.705 = 82		0.805 = 94		0.905 = 105		1.005 = 117			
0.110 = 13		0.210 = 24		0.310 = 36		0.410 = 48		0.510 = 59		0.610 = 71		0.710 = 83		0.810 = 94		0.910 = 106		1.010 = 117			
0.115 = 13		0.215 = 25		0.315 = 37		0.415 = 48		0.515 = 60		0.615 = 72		0.715 = 83		0.815 = 95		0.915 = 106		1.015 = 118			
0.120 = 14		0.220 = 26		0.320 = 37		0.420 = 49		0.520 = 60		0.620 = 72		0.720 = 84		0.820 = 95		0.920 = 107		1.020 = 119			

TAcY can be calculated by the following formula

$$T_{\text{Acy}} = (\text{Absorbance} / 0.0086) - 0.008$$

APPENDIX 4

TITLE: CRANBERRY PROCESSES AND PRODUCTS

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"EXPRESS MAIL" Mailing Label Number EL245470945US

Date of Deposit November 9, 1998

I hereby certify under 37 CFR 1.10 that this correspondence is being deposited with the United States Postal Service as "Express Mail Post Office To Addressee" with sufficient postage on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Pamara M. Scarlett
Pamela M. SCARLETT

Appendix 4

TITLE: TITRABLE ACIDITY MEASUREMENT

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I. Equipment

Fisher, Orion or Corning pH meter (or equivalent)
100 ml beakers
50 ml burette and holder
Magnetic stir bars
Magnetic stirrer
Waring blender
10 ml graduated pipette
Sartorius, Mettler, O'Haus or equivalent electronic balance,
readable to 0.01 g.

II. Reagents

Distilled water
Standardized sodium hydroxide (NaOH), .1 N
Buffer solutions (standard pH 7 and pH 4) for pH meter calibration
Saturated KCl solution
Electrode storage solution

III. Procedure

A. Setting Up

1. Electrode(s) should be stored in distilled water or an electrode storage solution before and after use (check manufacturer's guide.)
2. If using a half cell electrode set-up, make sure the pH half cell is filled with saturated KCl solution. Make sure the electrode junction(s) of both half cell and combination electrodes are clean.
3. Fill burette with standardized .1 N NaOH.

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B. Standardize pH meter

1. Turn on stirrer and following rinse with distilled water, insert electrode(s) in pH 7 buffer solution with a stir bar. (Make sure electrode(s) clear the stir bar.)
2. Turn pH meter on and read pH. With the calibration (or standardization) knob, set reading to pH indicated on the buffer bottle for the solutions temperature. Remove electrode(s) from buffer solution and rinse with distilled water. Next, insert electrode(s) in pH 4 buffer solution with a stir bar. Set pH reading to 4.00 using the percent slope or temperature knob. Remove electrode(s) from buffer and rinse with distilled water. Repeat these steps, making sure that the pH reads 7.00 and 4.00 respectively without using adjustment knobs. The pH meter is now standardized. Turn the pH meter back to off or stand-by.

NOTE: The names of the "adjustment knobs" on different pH meters vary from unit to unit. Please refer to owners manual for correct nomenclature.

C. Sample Preparation

1. Beverages - pipette 10 ml into a 100 ml beaker.
2. Sauces and viscous liquids (concentrates) -- weigh to the nearest hundredth approximately 10 g into a 100 ml beaker. Use Waring blender to homogenize sauces before weighing.
3. Add stir bar to sample and enough distilled water so that the bar will not touch the immersed electrodes.

D. Sample Analysis

1. Turn on stirrer and immerse electrodes. (Be sure electrodes clear stir bar.)
2. If using a graduated glass burette to titrate, make sure there are no air bubbles in the burette. If using an automatic digital burette, clear lines of any stagnant NaOH (discard), making sure no air bubbles are left in the burette.
3. Zero the burette before titrating.
4. Turn pH meter on. (See owner's manual if further clarification is required.)

5. Add NaOH until a pH of 8.1 is reached, signalling the end point of the titration.
6. Set pH meter to standby, turn off the stirrer and read the burette. Record reading.
7. Remove electrode(s) from sample, rinse with distilled water and replace in storage solution (distilled water or electrode storage solution).

E. Calculations

1. Percent titratable acidity (TA) as weight per volume (w/v) =
$$\frac{\text{mL NaOH used} \times \text{SF}^* \times \text{N of NaOH} \times 100}{10 \text{ mL (sample volume)}}$$
2. ‡ titratable acidity as weight per weight =
$$\frac{\text{mL NaOH used} \times \text{SF}^* \times \text{N of NaOH} \times 100}{\text{weight of sample (grams)}}$$

Standardization Factor - Values depend on acid being measured. Use table below. (Typically, use citric acid factor and report as such, unless otherwise specified.)

Acid	SF
Acetic	0.060
Citric	0.064
Malic	0.067
Tartaric	0.075

IV. Preparing .1 N NaOH

Purchase NaOH pellets from Fisher Scientific or equivalent. Dissolve 20g of NaOH (m.w = 40) with 5 L of distilled water. After adding water to pellets, allow mixture to stand overnight before standardizing. Be sure to label titrant container with titrant name, concentration of titrant (e.g. .1 N), date of preparation and initials of person who made up the solution.

OR

Purchase 0.1 N NaOH from Fisher Scientific or equivalent, and label as instructed above.

V. Standardizing NaOH

1. Shake solution well before testing.
2. Dilute 10 ml of 1 N HCl (purchased from Fisher Scientific or equivalent) to 100 ml in a volumetric flask.
3. Take 10 ml of the resulting .1 N HCl and to it add 30 ml of distilled water.
4. Titrate against NaOH to pH 8.1 on meter.

Calculation

$$\frac{.1 \text{ N HCl} \times 10 \text{ ml}}{\text{mL NaOH to reach 8.1 endpoint}} = \text{normality of NaOH}$$

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ATTACHMENT 1 - Volume of single strength cranberry juice (SSCJ) or Diluted Concentrate to Determine Color @ 7.5°Brix.

1. Determine °Brix of SSCJ
2. Using a graduated, wide-mouth, 5 mL pipette, transfer into a 100 mL volumetric flask the amount of juice indicated in the table below: (For volumes over 5.0 mL, a graduated 10 mL or 25 mL pipette is to be used.)

Sample °Brix	mLs of Sample	Sample °Brix	mLs of Sample	Sample °Brix	mLs of Sample	Sample °Brix	mLs of Sample
.5	40.0	2.7	7.4	4.9	4.0	7.1	2.8
.6	33.5	2.8	7.1	5.0	3.9	7.2	2.7
.7	28.7	2.9	6.9	5.1	3.9	7.3	2.7
.8	25.1	3.0	6.8	5.2	3.8	7.4	2.6
.9	22.3	3.1	6.4	5.3	3.7	7.5	2.6
1.0	20.1	3.2	6.2	5.4	3.7	7.6	2.6
1.1	18.2	3.3	6.0	5.5	3.6	7.7	2.5
1.2	16.7	3.4	5.9	5.6	3.5	7.8	2.5
1.3	15.4	3.5	5.7	5.7	3.5	7.9	2.5
1.4	14.3	3.6	5.5	5.8	3.4	8.0	2.4
1.5	13.4	3.7	5.4	5.9	3.3	8.1	2.4
1.6	12.5	3.8	5.2	6.0	3.3	8.2	2.4
1.7	11.8	3.9	5.1	6.1	3.2	8.3	2.4
1.8	11.1	4.0	5.0	6.2	3.2	8.4	2.3
1.9	10.5	4.1	4.8	6.3	3.1	8.5	2.3
2.0	10.0	4.2	4.7	6.4	3.1	8.6	2.3
2.1	9.5	4.3	4.6	6.5	3.0	8.7	2.2
2.2	9.1	4.4	4.5	6.6	3.0	8.8	2.2
2.3	8.7	4.5	4.4	6.7	2.9	8.9	2.2
2.4	8.3	4.6	4.3	6.8	2.9	9.0	2.2
2.5	8.0	4.7	4.2	6.9	2.8	9.1	2.1
2.6	7.7	4.8	4.1	7.0	2.8		

$$\text{mLs of sample} = \frac{(2.81 \text{ mLs}) (0.64269)}{\text{lbs. solids/gal. @ indicated } ^\circ\text{Brix}}$$

If °Brix of sample is less than 0.5 greater than 9.1, use above formula to determine volume to use.